Performance characteristics, Continued

Intra-assay precision

Samples with known Ms IL-6 concentration were assayed in replicates of 14 to determine precision within an assay.

Parameters	Sample 1	Sample 2	Sample 3
Mean (pg/mL)	54.9	106.2	313.6
SD	3.8	5.5	16.9
%CV	6.9	5.2	5.4

SD = Standard Deviation
CV = Coefficient of Variation

Inter-assay precision

Samples were assayed 42 times in multiple assays to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (pg/mL)	55.2	103.9	313.3
SD	4.4	6.0	20.3
%CV	8.0	5.8	6.5

SD = Standard Deviation
CV = Coefficient of Variation

Specificity

Buffered solutions of a panel of substances at 10,000 pg/mL were assayed with the Ms IL-6 kit. The following substances were tested and found to have no cross–reactivity: mouse IL-1β, IL-2, IL-4, IL-10, IFN-γ, TNF-α; rat IL-1β, IL-2, IL-4, IL-6, IFN-γ, TNF-α; human IL-2, IL-4, IL-6, IL-10, IL-12, IFN-γ, TNF-α and swine IL-8.

Recovery

The following table shows the average recovery when adding Ms IL-6 to the listed sample types.

Sample type	Average % Recovery
Serum	83
Citrate plasma	95
Culture medium containing 1% fetal bovine serum	106
Culture medium containing 10% fetal bovine serum	101

Linearity of dilution

Mouse serum and tissue culture medium containing 10% fetal bovine serum were spiked with Ms IL-6 and serially diluted in Standard Diluent Buffer over the range of the assay. Linear regression analysis of samples versus the expected concentration yielded a correlation coefficient of 0.99 in both cases.

	Serum			Cell Lysate		
Dilution	Measured (pg/mL)	Expected (pg/mL)	% Expected	Measured (pg/mL)	Expected (pg/mL)	% Expected
Neat	916	—	—	415	—	—
1/2	423	458	92	220	208	106
1/4	221	229	97	104	104	100
1/8	104	115	90	54	52	104
1/16	48	57	84	27	26	104

Expected Values

Ten sera and ten plasma (citrate) samples were evaluated in this assay.

- The values for sera ranged from 0–20 pg/mL (mean = 4.4 pg/mL).
- The values for plasma ranged from 0–39 pg/mL (mean = 6.1 pg/mL).

Mouse splenocytes were cultured under the following conditions and the culture supernatants were assayed for released Ms IL-6.

Sample	Average (pg/mL)
Con-A (5 µg/mL) 6 hours	185
PHA (5 µg/mL), LPS (25 µg/mL) 4 hours	154
PHA (5 µg/mL), LPS (25 µg/mL) 24 hours	97
PMA (50 ng/mL) Ionophore (250 ng/mL) 12 hours	7

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Product label explanation of symbols and warnings

	Manufacturer	REF	Catalog Number	LOT	Batch code		Consult instructions for use		Use by		Temperature limitation		Caution, consult accompanying documents

novex by life technologies™

Mouse IL-6 ELISA Kit

Catalog. no. KMC0061 (96 tests), KMC0062 (192 tests), KMC061C (480 tests)

Pub. Part no. PR030 MAN0003388 Rev. 2.00

Description

The Mouse IL-6 ELISA Kit is a solid-phase sandwich Enzyme Linked Immunosorbent Assay (ELISA). This assay is designed to detect and quantify the level of Mouse Interleukin-6 (Ms IL-6) in mouse serum, plasma, buffered solution, or cell culture medium. The assay will recognize both natural and recombinant Ms IL-6.

IL-6 is a 21–28 kDa glycoprotein composed of 184 amino acids produced by lymphocytes, monocytes, fibroblasts, keratinocytes, endothelial cells, mesangial cells, astrocytes, bone marrow stroma, and tumor cells. At the protein level, mouse IL-6 shows 42% homology to human IL-6, while mouse and rat are 93% identical. IL-6 plays a major role in the regulation of cell growth, hematopoiesis, and inflammation. IL-6 induces maturation of B-cells into antibody–secreting plasma cells and it co-stimulates T-cell growth and cytotoxic T-cell differentiation. IL-6 increases IL-2 receptor production in T-cells and induces production of IL-2. IL-6 is the major inducer of acute phase reactions in response to inflammation or tissue injury, and with IL-1β and TNF-α, IL-6 induces synthesis of acute phase proteins by hepatocytes.

Contents and storage

The components included in the ELISA kit are listed below. Upon receipt, store the kit at 2 to 8°C.

Components	Cat. no. KMC0061 96 tests	Cat. no. KMC0062 192 tests	Cat. no. KMC061C 480 tests
Ms IL-6 Standard (recombinant Ms IL-6), lyophilized, contains 0.1% sodium azide. Refer to vial label for quantity and reconstitution volume.	2 vials	4 vials	10 vials
Standard Diluent Buffer, contains 0.1% sodium azide	25 mL	2 × 25 mL	5 × 25 mL
Antibody Coated Wells, 12 × 8 Well Strips	1 plate	2 plates	5 plates
Ms IL-6 Biotin Conjugate, (Biotin-labeled anti-IL-6), contains 0.1% sodium azide	11 mL	2 × 11 mL	5 × 11 mL
Streptavidin-HRP (100X), contains 3.3 mM thymol	0.125 mL	2 × 0.125 mL	5 × 0.125 mL
Streptavidin HRP Diluent, contains 3.3 mM thymol	25 mL	25 mL	3 × 25 mL
Wash Buffer Concentrate (25X)	100 mL	100 mL	2 × 100 mL
Stabilized Chromogen, Tetramethylbenzidine (TMB)	25 mL	25 mL	3 × 25 mL
Stop Solution	25 mL	25 mL	3 × 25 mL
Plate Covers, adhesive strips	3	6	15

CAUTION! This kit contains materials with small quantities of sodium azide. Sodium azide reacts with lead and copper plumbing to form explosive metal azides. Upon disposal, flush drains with a large volume of water to prevent azide accumulation. Avoid ingestion and contact with eyes, skin and mucous membranes. In case of contact, rinse affected area with plenty of water. Observe all federal, state, and local regulations for disposal.

Materials required but not provided

- Distilled or deionized water
- Microtiter plate reader (at or near 450 nm) with software
- Plate washer–automated or manual (squirt bottle, manifold dispenser, or equivalent)
- Calibrated adjustable precision pipettes and glass or plastic tubes for diluting solutions

Before starting

Review the **Procedural guidelines** and **Plate washing directions** in the *ELISA Technical Guide* available at **www.lifetechnologies.com/manuals** for details prior to starting the procedure.

Note: Reagents are lot-specific. Do not mix or interchange different reagent lots from various kit lots.

Dilute wash buffer

1. Allow the Wash Buffer Concentrate (25X) to reach room temperature and mix to redissolve any precipitated salts.
2. Dilute 1 volume of the Wash Buffer Concentrate (25X) with 24 volumes of deionized water (e.g., 50 mL may be diluted up to 1.25 liters, 100 mL may be diluted up to 2.5 liters). Label as Working Wash Buffer.
3. Store the concentrate and the Working Wash Buffer in the refrigerator. Use the diluted buffer within 14 days.

Prepare Streptavidin-HRP solution

Note: Prepare Streptavidin-HRP within 15 minutes of usage.

The Streptavidin-HRP (100X) is in 50% glycerol, which is viscous. To ensure accurate dilution:

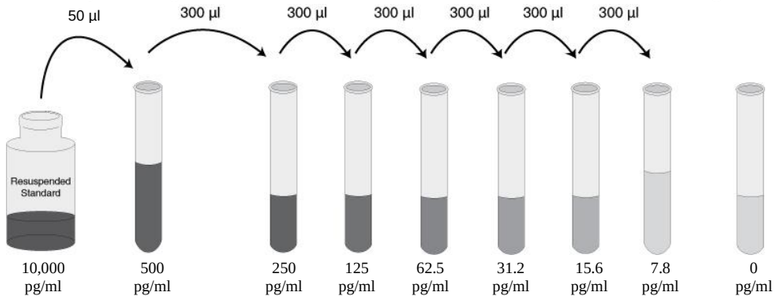
1. For each 8-well strip used in the assay, pipet 10 µL Streptavidin-HRP (100X) solution, wipe the pipette tip with a clean absorbent paper to remove any excess solution, and dispense the solution into a tube containing 990 µL of HRP Diluent. Mix thoroughly.
2. Return the unused Streptavidin-HRP (100X) solution to the refrigerator.

Dilute the standards

Note: One nanogram of recombinant Ms IL-6 equals 1260 arbitrary units of WHO reference preparation 93/730 (NIBSC, Hertfordshire, UK, EN6 3QG). Use glass or plastic tubes for diluting standards.

1. Reconstitute Ms IL-6 Standard to 10,000 pg/mL with Standard Diluent Buffer. Refer to the standard vial label for instructions. Swirl or mix gently and allow the contents to sit for 10 minutes to ensure complete reconstitution. Add 50 µL of the reconstituted standard to a tube containing 950 µL Standard Diluent Buffer. Label as 500 pg/mL Ms IL-6. Use the standard within 1 hour of reconstitution.
2. Add 300 µL Standard Diluent Buffer to each of 6 tubes labeled as follows: 250, 125, 62.5, 31.2, 15.6, and 7.8 pg/mL of Ms IL-6.
3. Make serial dilutions of the standard as described below in the dilution diagram. Mix thoroughly between steps.

Discard any remaining reconstituted standard. Return the Standard Diluent Buffer to the refrigerator.



Sample preparation guidelines

- Collect samples in pyrogen/endotoxin-free tubes.
- Samples should be frozen at –80°C if not analyzed shortly after collection. Avoid multiple freeze-thaw cycles of frozen samples. Thaw completely and mix well prior to analysis.
- When possible, avoid use of badly hemolyzed or lipemic sera. If large amounts of particulate matter are present in the sample, centrifuge or filter sample prior to analysis.

ELISA procedure

Allow all reagents to reach room temperature before use. Mix all liquid reagents prior to use. **Total assay time is 3.5 hours.**

IMPORTANT! Perform a standard curve with each assay.

Determine the number of 8-well strips required for the assay. Insert the strips in the frames for use. Re-bag any unused strips and frames, and store at 2 to 8°C for future use.

Bind antigen

1. Add 100 µL of standards, TC samples or controls to the appropriate microtiter wells. For sera and plasma samples, add 50 µL of standard diluents to the appropriate microtiter wells followed by 50 µL of sample.
2. Cover the plate with plate cover and incubate for 2 hours at room temperature.
3. Thoroughly aspirate the solution and wash wells 4 times with diluted Wash Buffer.

Add detector antibody

4. Add 100 µL Ms IL-6 Biotin Conjugate solution into each well except chromogen blanks.
5. Cover the plate with plate cover and incubate for 30 minutes at room temperature.
6. Thoroughly aspirate the solution and wash wells 4 times with diluted Wash Buffer.

Add Streptavidin-HRP

7. Add 100 µL Streptavidin-HRP (see page 2) into each well except the chromogen blanks.
8. Cover the plate with plate cover and incubate for 30 minutes at room temperature.
9. Thoroughly aspirate the solution and wash wells 4 times with diluted Wash Buffer.

Add chromogen

10. Add 100 µL Stabilized Chromogen to each well. The substrate solution will begin to turn blue.
11. Cover the plate with plate cover and incubate for 30 minutes at room temperature **in the dark**.

Note: TMB should not touch aluminum foil or other metals.

Add stop solution

12. Add 100 µL Stop Solution to each well. Tap side of the plate gently to mix. The solution in the wells changes from blue to yellow.

Read the plate and generate the standard curve

1. Read the absorbance at 450 nm. Read the plate within 2 hours after adding the Stop Solution.
2. Use curve-fitting software to generate the standard curve. A four parameter algorithm provides the best standard curve fit. Optimally, the background absorbance may be subtracted from all data points, including standards, unknowns and controls, prior to plotting.
3. Read the concentrations for unknown samples and controls from the standard curve. Multiply value(s) obtained for sample(s) by the appropriate factor to correct for the sample dilution.

   Note: Dilute samples producing signals greater than that of the highest standard in Standard Diluent Buffer and reanalyze. Multiply the concentration by the appropriate dilution factor.

Performance characteristics

Standard curve (example)

The following data were obtained for the various standards over the range of 0–500 pg/mL Ms IL-6.

Standard Ms IL-6 (pg/mL)	Optical density (450 nm)
500	2.85
250	1.58
125	0.82
62.5	0.43
31.2	0.25
15.6	0.16
7.8	0.12
0	0.09

Sensitivity

The minimum detectable concentration of Ms IL-6 is <3 pg/mL. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 30 times.